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10/539,954	06/17/2005	Oliver Schmitz	13195-00006-US	8865

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EXAMINER	
CHOWDHURY, IQBAL HOSSAIN	
ART UNIT	**PAPER NUMBER**
1652	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/539,954	SCHMITZ ET AL.	
	Examiner	Art Unit	
	IQBAL H. CHOWDHURY	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *18 December 2008*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,4,7-17,26 and 27* is/are pending in the application.

4a) Of the above claim(s) *27* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,4,7-17 and 26* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Claims 1, 4, 7, 9-17, 26 and 27 are currently pending.

In response to a previous Office action, a final action (mailed on February 3, 2009), Applicants filed a response and amendment on December 18, 2008, amending claims 1, 4 and 26, and cancelling claims 2, 5 and 8 is acknowledged. Claim 27 remain withdrawn as encompassing non-elected invention.

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 12/18/2008 has been entered.

Claims 1, 4, 7, 9-17 and 26 are under consideration.

Applicants' arguments filed on December 18, 2008, have been fully considered but are not deemed persuasive to overcome some of the rejections previously applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

Maintained-Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the

art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

The previous rejection of claims 1, 4, 7 and 9-17 under 35 U.S.C. 112, first paragraph on scope of enablement is maintained. This rejection has been discussed at length in the previous office action. The rejection is maintained for the following reasons.

The specification, while being enabling for a process for preparing amino acid methionine in transgenic organism, wherein the process comprises introduction of a nucleic acid sequence of SEQ ID NO: 1 encoding a threonine-degrading protein i.e. threonine aldolase of SEQ ID NO: 2 from S. cerevisiae, does not reasonably provide enablement for a process for preparing amino acids, wherein the process comprises introduction of a nucleic acid sequence encoding a polypeptide, which is 85% identical to SEQ ID NO: 2 having threonine-degrading activity (claims 1). The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, **to make and/or use** the invention commensurate in scope with these claims.

Arguments/Response:

Applicants argue that the finality of the present action be withdrawn because the finality of the present Office Action is inappropriate, since, the Examiner in the enablement rejection in the Final Office Action cites a new reference, i.e. Guo *et al.,* to support the rejection. Pursuant to MPEP § 706.07(a), a second or any subsequent action on the merits in any application will not be made final if it includes a rejection, on

newly cited art. Because this is a new reference to support the enablement rejection, the action should not have been made final.

This is not found persuasive because Guo et al. reference was used as a supporting reference against the arguments by the applicants in 112(1st) rejection, not as a primary reference for any rejection such as 102 or 103. Thus, the finality of the office action was proper.

Applicants also argue that there has never been a requirement that every species encompassed by a claim must be disclosed or exemplified. There is no requirement for Applicants to produce and test all possible variants. Applicants further argue that the standard for enablement does not turn on whether or not a large amount of screening is required. Under the applicable law, the test for "undue experimentation" is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine.

The Examiner agrees that applicants do not need to disclose every species or test all possible variants. However, the proteins which are 85% identical to SEQ ID NO: 2 comprises many mutants, variants or fragments, even different enzymes as well as inactive mutants, which in turn warrants many undue experimentation and thus, searching for an active mutants from extremely large number of species is extremely difficult for one of skilled artisan and one of skilled artisan would not know how to make the claimed invention without knowing the structure of the protein used in the claimed method.

Applicants further argue that Guo et *at.* (hereinafter "Guo") reference do not support the enablement rejection, and also argue that the applicants disagree with the Examiners contention that Guo provides a formula for calculating a percentage of active mutants, and from a calculation of potential mutants based on this formula that guidance is not provided and that undue experimentation would be required. Applicants strongly disagree with the Examiner interpretation of this reference. Furthermore, applicants argue that Guo discloses that the components of formula [I] and [2] are solved after experimental determination (see Guo, page 9206, left column, 3rd line following formula [11).

This is not found persuasive because Guo et al. indeed provide a formula for calculating a percentage of active mutants by sequencing AAG proteins from different mutation library and extrapolated that experimental data for a probability of protein inactivation using said formula based on the number of mutations of a protein (see abstract, p9205, right column, paragraph 1, 5, p9206 left column 1-5). Regarding the arguments that the components of formula I and II, which can be solved after experimental determination, the examiner disagrees with the applicant's contention because Guo et al. gathered experimental data from sequencing many proteins of AAG and deduced a formula by which a probability of other protein inactivation can be determined by using the formula determined from AAG. Guo et al. further provide the support for their formula by citing the results of Markiewicz and coworkers (1994) and Eyre-Walker and Keightley et al. (1999) (see p9206, right column, paragraph 2 of Guo et al. reference). Guo et al. state that Markiewicz and coworkers examined 12 or 13

different amino acid substitutions at each residue across 90% of the 360 amino acid E. coli Lac repressor protein, which corresponded to two or three nucleotide changes per codon. Guo et al. analyzed Markiewicz and coworkers data and found that 1380 single mutants were inactive, which is 20% of total 4049 mutants examined that yielded an x-factor of the lac repressor gene 34% similar to Guo et al. findings on AAG gene. Similarly, Guo et al. state that Eyre-Walker and Keightley et al. calculated the percentage of deleterious substitution mutations that were eliminated from human lineage by purifying selection, wherein examined synonymous non-synonymous substitution rates from coding regions of 46 homologous proteins from human and chimpanzees, where Eyre-Walker and Keightley concluded that at least 38% of spontaneous mutations in the human lineage were sufficiently deleterious (see p9206, right column, paragraph 2 of Guo et al. reference). Guo et al. suggests that the data from Markiewicz and coworkers and Eyre-Walker and Keightley et al. experiments are within a range of similar x-factors over the length of diverse proteins as obtained by Guo et al. (p9206, right column, paragraph 2).

Applicants further argue that Table 1 provides results using the human DNA repair enzyme 3-methyladenine DNA glycosylate (AAG) and generated three different AAG mutant libraries. From the library with the low degree of mutation having a size of 2 x $10^{8,}$ only 20 mutants were sequenced showing that 1 mutant (5%) had no amino acid changes, 4 mutants (20%) showed one amino acid change, 8 mutants (40%) showed two amino acid changes and 3 mutants (15%) showed four amino acid changes within AAG. Based on the data of the library with the low mutation degree and the 20 mutants

which have been sequenced, Guo has extrapolated a **so-called x-factor** of 0.39 (39%) and including all three libraries an average x-factor of 0.34 (34%).

This is not found persuasive because Guo et al. indeed provides a formula for calculating a percentage of active mutants by sequencing AAG proteins from different mutant library and extrapolated that experimental data for a probability of protein inactivation using said formula and obtained x-factor of 34% with statistical significance and extrapolated that findings in a formula for calculating active mutants, which is also supported by the findings of Markiewicz and coworkers (1994) and Eyre-Walker and Keightley et al. (1999), which is applicable for any proteins as claimed by Guo et al. which is published in a well reputed peer reviewed Journal PNAS. Thus, the calculated x-factor is not "so called x-factor" but derived from experimental data, with mathematical interpretation (exponential) for number of mutations of a specific protein having specific amino acids changes.

Applicants further argue that the Examiner interpretation of the percentage of random substitution mutation which inactivates AAG is 34% is incorrect and further state that Figure 1 of Guo clearly shows that only 34 out of 299 amino acids of AA (cannot be replaced without loss of activity meaning that 299 minus 34 amino acids can be replaced meaning that at least (299-34)/299 = 9I% of single mutations result in mutants which are still active. This result is diametrically opposed to the assumption of the Examiner.

This is not found persuasive because nowhere in the Guo et al. reference recites that 34 out of 299 amino acids of AAG cannot be replaced without loss of activity. Besides, Guo et al. clearly indicated from experimental data that the percentage of random substitution mutation which inactivates AAG is 34% (see Table 1). The Examiner agrees that this generic formula may not applicable to every protein but it is applicant's burden to provide evidence in the contrary.

Regarding the arguments that the Board of Patent Appeal and Interference found enable for Ex parte Sun is not persuasive because Ex Parte Sun deals with different protein which cannot be compared with the instant application because each application has to be examined on its own merits and cannot be compared with others.

As discussed previously, Claims 1, 7, and 9-17 are so broad as to encompass a process for preparing amino acids in a transgenic organism, wherein the process comprises introduction of a nucleic acid sequence encoding any threonine-degrading protein, which is at least 85% identity to SEQ ID NO: 2, (15% non-identity for claim 1), i.e. applicants are claiming a method of using a polypeptide, wherein 58 amino acids are different in any combination out of 387 amino acids protein. Claims still read on using any nucleic acid having threonine degrading activity and the consensus sequence does not give any structural feature of said consensus sequence because said sequence has 56 unknown amino acids out of 62, which is enormously broad that does not provide any information to predict structural feature of the polypeptide having threonine degrading activity. Claims as written interprets any polypeptide, which comprises many mutants, variants and fragments having threonine degrading activity.

One of ordinary skilled in the art would not know how to make the claimed invention without structural feature of the claimed nucleic acid molecule encoding polypeptide used in the claimed method which would require undue experimentation. The scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of nucleic acid sequences encoding any threonine-degrading protein having at least 85% identity to SEQ ID NO: 2 (claim 1), which includes many mutants and variants used in the claimed method. However, in this case the disclosure is limited to the nucleotide and encoded amino acid sequence of only one threonine degrading protein i.e. threonine aldolase of SEQ ID NO: 2 encoded by SEQ ID NO: 1 used in the claimed method.

While methods to produce variants of a known sequence such as site-specific mutagenesis, random mutagenesis, etc. are well known to the skilled artisan, producing variants useful as threonine aldolase having threonine degrading activity requires that one of ordinary skill in the art know or be provided with guidance for the selection of which of the infinite number of variants have the activity. Without such guidance one of ordinary skill would be reduced to the necessity of producing and testing all of the virtually infinite possibilities. For the rejected claims, this would clearly constitute **undue** experimentation. For example, Guo et al. (Protein tolerance to random amino acid change, Proc Natl Acad Sci U S A, 2004 Jun 22; 101(25): 9205-10, Epub 2004 Jun 14) teach that the percentage of random single substitution mutations which inactivate a protein for the protein 3-methyladenine DNA glycosylase is 34% and that this number appears to be consistent with other studies in other proteins as well. Guo et al. further

show in Table 1 that the percentage of active mutants for multiple mutants appears to be exponentially related to this by the simple formula $(.66)^x$ X 100%, where x is the number of mutations introduced. Applying this estimate to the instant protein 85% identity allows up to 58 mutations within the 387 amino acids of SEQ ID NO: 2 and thus only $(.66)^{58}$ X 100% or 3.4 x 10^{-9}% (i.e. $\cong$ 1 in 2.9 billions) of random mutants having 85% identity would be active. Similarly at 95% identity 3.7 x 10^{-2}% (i.e. 1 in 2683). Current techniques (i.e., high throughput mutagenesis and screening techniques) in the art would allow for finding a few active mutants within several hundred of inactive mutants as is the case for the claims limited to 95% identity (despite even this being an enormous quantity of experimentation that would take a very long time to accomplish) but finding a few mutants within many billions or more as in the claims to 85% or less identity would not be possible. While enablement is not precluded by the necessity for routine screening, if a large amount of screening is required, the specification must provide a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. Such guidance has **not** been provided in the instant specification.

Sufficient guidance has **not** been provided in the instant specification or in the prior art as at best art teaches to avoid changes of 5% of the structure of SEQ ID NO: 2 but does little to suggest what changes would be successful particularly for those enzymes having the substantial number of alterations necessary to produce a protein having 85% identity to SEQ ID NO: 2.

Applicants have not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims broadly including a process for preparing an amino acid in a transgenic organism, wherein the process comprises introduction of any nucleic acid sequence encoding any threonine-degrading protein, or any nucleic acid sequence encoding any protein having at least 85% identity to SEQ ID NO: 2. The scope of the claims must bear a reasonable correlation with the scope of enablement (In re Fisher, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of a process for preparing amino acids in a transgenic organism by introducing of a nucleic acid sequence encoding any threonine-degrading protein, or any nucleic acid sequence encoding any protein having at least 85% identity to SEQ ID NO: 2 having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See In re Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Therefore, the rejection is maintained.

Maintained-Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless -

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

The previous rejection of Claims 1, 7, 10, 14-16 and 26 under 35 U.S.C. 102(b) as being anticipated by Monschau et al. (Threonine aldolase overexpression plus threonine supplementation enhanced riboflavin production in Ashbya gossypii, Appl Environ Microbiol. 1998 Nov; 64(11): 4283-90, see IDS) is maintained. This rejection has been discussed at length in the previous office action. The rejection is maintained for the reasons as discussed previously and for the following reasons.

The instant claims are drawn to a process for producing amino acid such as methionine, homoserine or lysine in transgenic organism including microorganism, wherein said microorganism is transformed with a vector comprising a gene encoding an enzyme having threonine degrading activity.

Monschau et al. teach a method of producing L-amino acid glycine in a fungal strain Ashbya gossypii comprising and overexpressing a gene encoding threonine aldolase from S. cerevisiae, which degrade threonine, which is 99.8% identical to SEQ ID NO: 2, inherently a threonine degrading enzyme, wherein the process produces amino acid glycine. Monschau et al. further teach that the threonine degrading protein (threonine aldolase) comprises consensus sequences of SEQ ID NO: 27 and 28 (claim 2), which are 100% identical to SEQ ID NO: 27 and 28 of the instant application. All microorganisms including filamentous fungus inherently produce L-amino acids including methionine, homoserine or lysine, as these amino acids are necessary for growth of said microorganism.

Arguments/Response:

Applicants argue that the claims have been amended without disclaimer or prejudice and the subject matter of claims 5 and 8 has been incorporated into claim 1, and thus also into the claims dependent therefrom. Since claim 8 was not included in the rejection and the subject matter of claim 8 is incorporated into the claims as amended, this rejection is believed to be rendered moot. Because Monschau does not teach every limitation of the claims, Monschau does not anticipate the claims as amended.

This is not found persuasive because claim 5 was included in the previous rejection, which comprises the limitation of "a nucleic acid molecule, which is 70% identical to SEQ ID NO: 2 with a negligible reduction in the threonine degrading activity of SEQ ID NO: 2", which is taught by Monschau et al. Indeed Monschau et al. teach GLY1 gene encoding threonine aldolase, which is 99.8% identical to SEQ ID NO: 2, inherently a threonine degrading enzyme. The limitation of claim 8 was only "the amino acid is isolated from transgenic organism, culture media or transgenic organism and culture media", which is also taught by Monschau et al. Monschau et al teach expression of GLY1 gene, which is 99.8% identical to SEQ ID NO: 2, inherently a threonine degrading enzyme in M13, a S. cerevisiae strain (p. 4283, line 6-7, abstract) results in expression of threonine aldolase with specific activity of 25 mU/mg protein in S. cerevisiae strain M13 that is glycine auxotroph strain. Since, the gene of the reference is 99.8% identical to SEQ ID NO: 2, inherently a threonine degrading enzyme and Monschau et al. indeed teach expression of a plasmid Yep352, which encodes GLY1 gene and expressed in YM13, a Saccharomyces cerevisiae strain (see p4288,

paragraph 3, line 11-16). Furthermore, Monschau et al. teach harvesting the transgenic microorganism to take dry weight of said transgenic microorganism (see p4284, Col 2, paragraph 6), which reads on isolating the amino acids.

Therefore, Monschau et al. anticipate claims 1, 7, 10, 14-16 and 26 of the instant application and the rejection is maintained as discussed.

Conclusion

Status of the claims:

Claims 1-2, 4-5, 7-17, and 26-27 are pending.

Claim 27 is withdrawn.

Claims 1, 4, 7-17, and 26 are rejected.

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Iqbal Chowdhury, whose telephone number is 571-272-8137. The examiner can normally be reached on 9:00-5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Andrew Wang can be reached on 571-272-0811. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Iqbal Chowdhury, Patent Examiner
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